

# Microscopic Mass Transfer and Its Influence on Microbial Reduction of U(VI)

Chongxuan Liu<sup>1</sup>, Zheming Wang<sup>1</sup>, John M. Zachara<sup>1</sup>, James K. Fredrickson<sup>1</sup>, Byong-Hun Jeon<sup>2</sup>, Paul D. Majors<sup>1</sup>, James P. McKinley<sup>1</sup>, and Steve M. Heald<sup>1,3</sup>

<sup>1</sup>Pacific Northwest National Laboratory, Richland, WA 99352; <sup>2</sup>Yonsei University, Kangwon-Do, Korea 220-710; <sup>3</sup>Argonne National Laboratory, Argonne, IL 60439



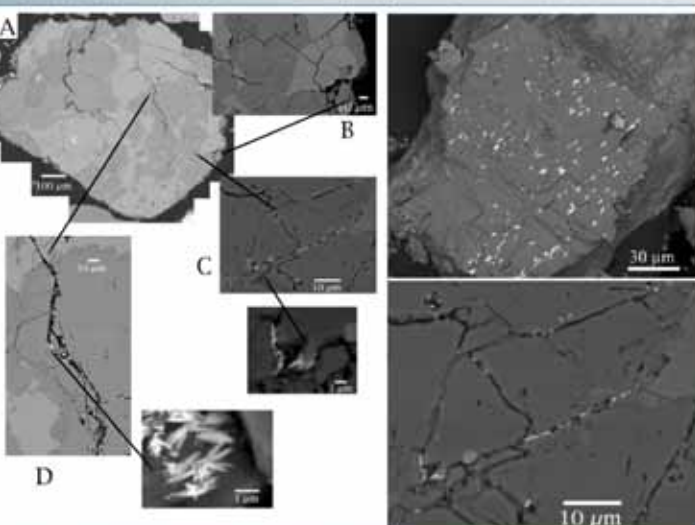
## Background

In contaminated subsurface sediments, U(VI) resides in both intergrain (where active water flow occurs) and intragrain domains (where static water resides). Dissimilatory metal reducing bacteria (DMRB) can reduce aqueous (intergrain) U(VI) to U(IV) under anoxic conditions yielding an insoluble precipitate [U(IV)O<sub>2</sub>]. Intragrain U(VI) becomes bioavailable if it dissolves and diffuses to the intergrain domain, or if the DMRB release soluble reductants that can diffuse to, and react with intraparticle U(VI) precipitates.

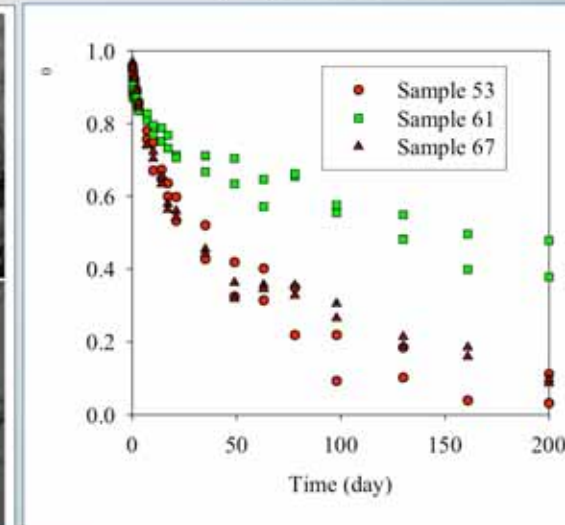
Microscopic and spectroscopic analyses of uranium-contaminated sediments from Hanford have revealed that U(VI) often exists as a precipitate within intragrain domains of sediment clasts. Intragrain U(VI) dissolves slowly into undersaturated pore water with kinetics limited by the mass transfer from intragrain domains to bulk solution.

This research investigated the microscopic mass transfer process and its effects on the microbial reduction of intragrain U(VI). The research has used Hanford sediments, but the resulting understanding and models are relevant to the Oak Ridge FRC, where a critical issue is the long term diffusion of U(VI) from a fine-grained saprolite matrix that is physically inaccessible to DMRB.

### SEM images of U(VI)-contaminated grains from Hanford 200 area



### Dissolution kinetics of intragrain U(VI) precipitates



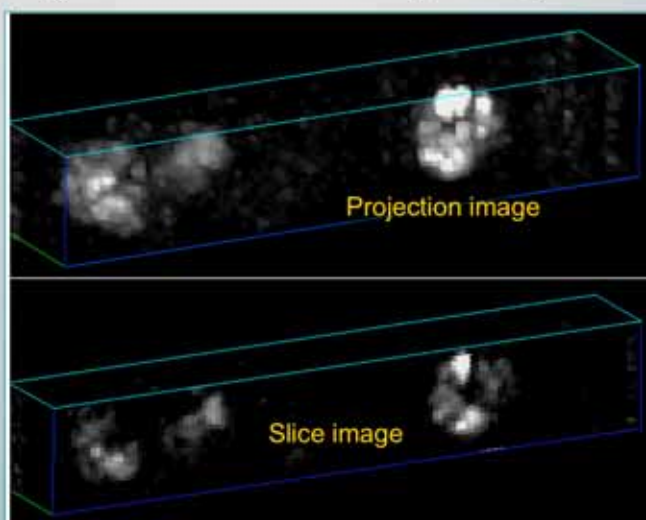
U(VI) precipitates (white) distributed within plagioclase microfractures and cleavages.

Dissolution rates of intraparticle U(VI) precipitates were slow (pH 9.2 and total CO<sub>2</sub> = 20 mM).

## Characterization of Mass Transfer Process

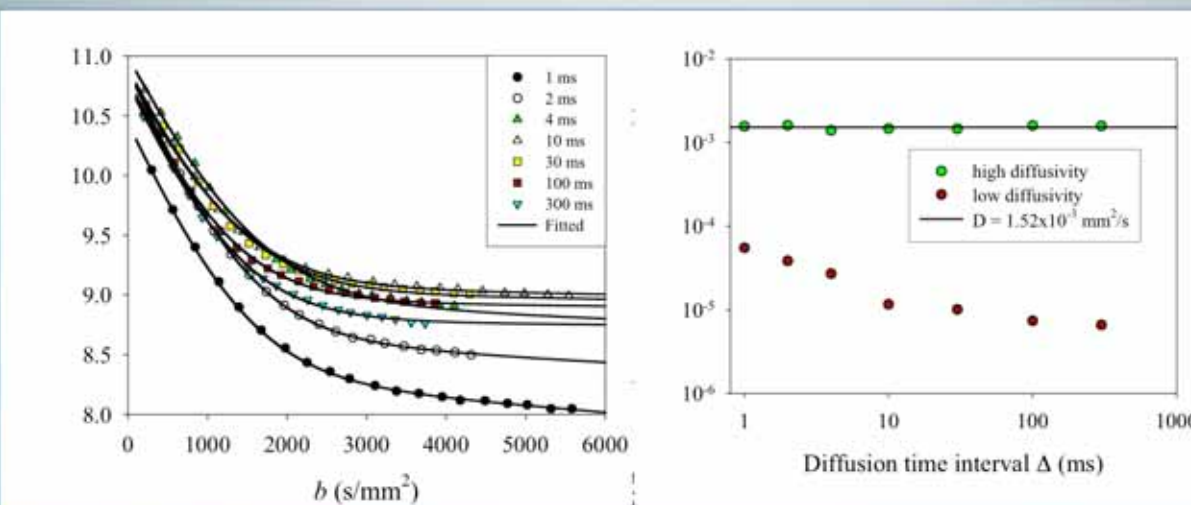
An NMR-PGSE approach was developed to characterize the microscopic mass transfer process at the grain scale and measure apparent intragrain <sup>1</sup>H<sub>2</sub>O diffusivity. The <sup>1</sup>H<sub>2</sub>O diffusivity was then used to calculate intragrain tortuosity.

### NMR images of water molecules on grain surfaces and intragrain regions



3D NMR images showing water as <sup>1</sup>H (white) on the surface of and within granitic lithic fragments immersed in a fluorinated oil which is invisible to NMR. The measured apparent diffusivity (plots below) was determined by water movement on the surface of and within the particle grains. (top: projection image; bottom: slice image).

### Measurements of intraparticle <sup>1</sup>H<sub>2</sub>O diffusion



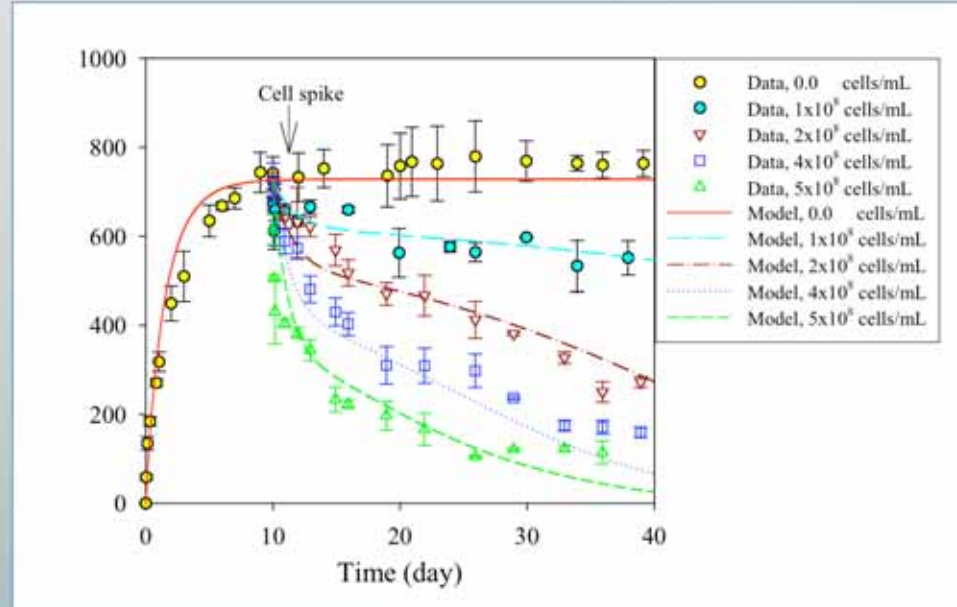
(Left) At a given constant diffusion time ( $\Delta t$ ), the echo intensity of <sup>1</sup>H spins decayed with increasing strength of NMR pulse field gradient (represented by parameter  $b$ ). Two combined exponential functions were needed to fit the decay curves, suggesting that <sup>1</sup>H<sub>2</sub>O diffused at two rates.

(Right) Intragrain diffusivities were calculated from the echo intensity curves (left) as a function of diffusion time ( $\Delta t$ ).

## Coupling of U(VI) Dissolution, Diffusion, and Microbial Activity

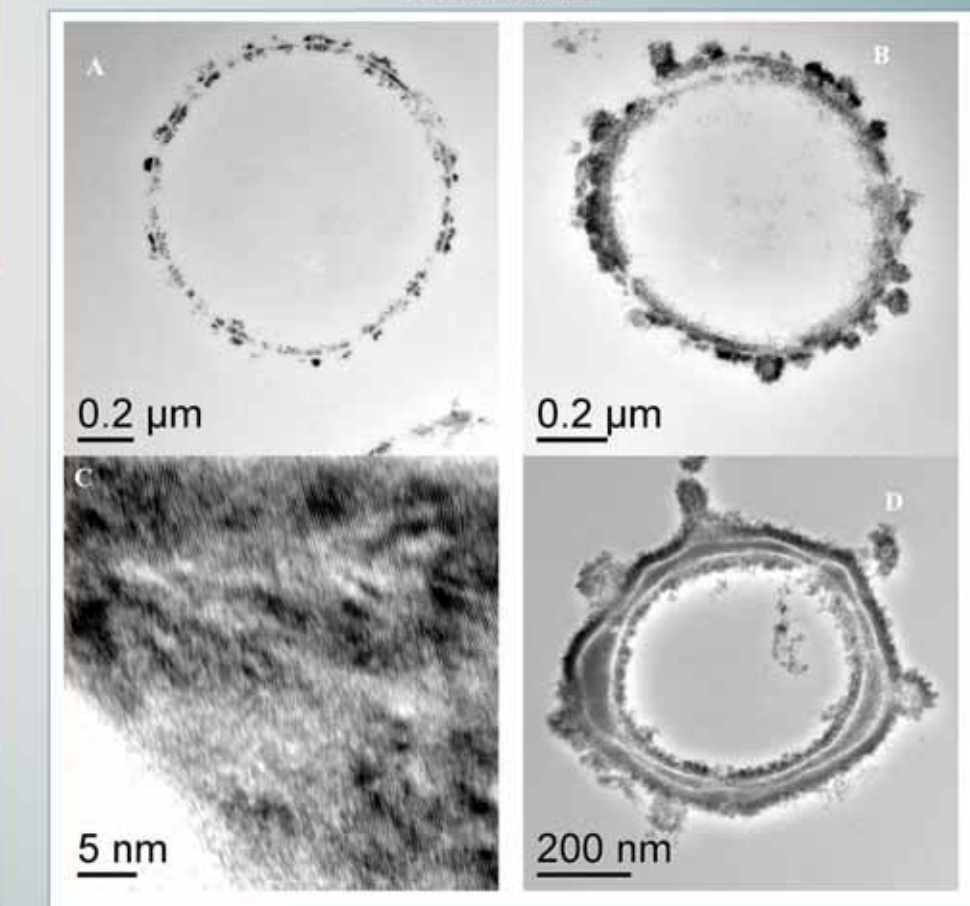
The microbial reduction of synthetic Na-boltwoodite embedded in alginate beads was studied to evaluate the coupling of U(VI) dissolution, diffusion, and microbial reduction of intragrain U(VI). LIFS imaging and spectroscopy, and TEM analyses were used to monitor the reduction reaction. The results indicated that U(VI) had to dissolve and diffuse out of intragrain regions before it was microbially reduced.

### Dissolution and microbial reduction of synthetic Na-boltwoodite

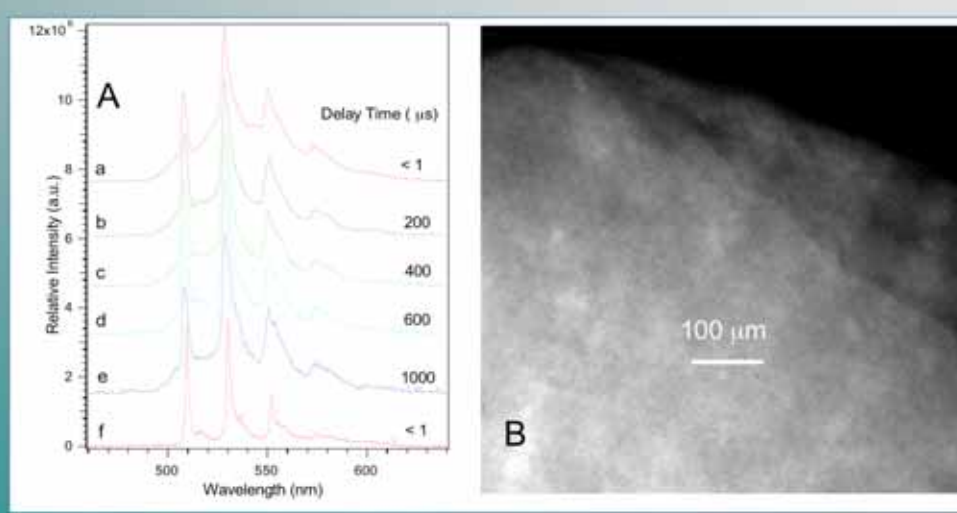


Microbial reduction of intragrain Na-boltwoodite was investigated as a function of bacterial cell concentration (left). The experimental results (symbols) were well described by the sequential coupling of intragrain U(VI) dissolution/diffusion, and microbial reduction of dissolved U(VI). Individual models and their parameters were independently determined.

### TEM images of cell surfaces and periplasm after U(VI) bioreduction



### LIFS spectra and image of intragrain U(VI) after microbial reduction



Residual U(VI) within alginate beads is Na-boltwoodite (A) after microbial reduction. The LIFS signal intensity (white, B) decreases toward the edges. Oxidative treatment of the beads with H<sub>2</sub>O<sub>2</sub> did not change the LIFS signal intensity and spectra, suggesting that intraparticle U(IV) was not present.

UO<sub>2</sub> accumulated on cell surfaces and in the periplasm. The accumulation increased with time. Sample A was collected at 2 days, sample B and C were collected at 7 days, and sample D was collected after 100 days of incubation.

## Objectives

- Characterize and develop numerical models to describe the microscopic mass transfer process in intragrain domains of Hanford sediment.
- Identify and characterize biogeochemical strategies used by DMRB to access intragrain U(VI) by representative dissimilatory reducing bacteria.
- Evaluate the coupling of dissolution kinetics, uranyl speciation, mass transfer, and microbial activities in the microbial reduction of intragrain U(VI) precipitates.

## Methods

Intragrain mass transfer phenomena and values of tortuosity within granitic lithic fragments, which are primary hosts of U(VI) precipitates in contaminated Hanford sediments, were characterized using a nuclear magnetic resonance, pulse gradient spin-echo (NMR-PGSE) technique. The NMR-PGSE results, intraparticle pore structure and porosity characterized from SEM, and macroscopic results of U(VI) precipitate dissolution were collectively used to develop a microscopic diffusive mass transfer model to describe coupled intraparticle U(VI) dissolution/precipitation and mass transfer.

The influence of mass transfer on microbial reduction was evaluated using both contaminated Hanford sediments and synthetic beads containing a synthetic uranyl-silicate (Na-boltwoodite) that was observed in the contaminated sediments. *S. ondesidensis* [MR-1 wild type and OmcA(AB) mutant] was used as model bacterium. Microscopic and spectroscopic techniques, such as XRM, SEM, TEM, and LIFS, were used to provide insights of microbial reduction mechanisms.

A model that coupled dissolution, uranyl speciation, mass transfer, and bacterial activity was parameterized from experiments that isolated these processes. The coupled model was applied to experiments where multiple, interactive processes had occurred.

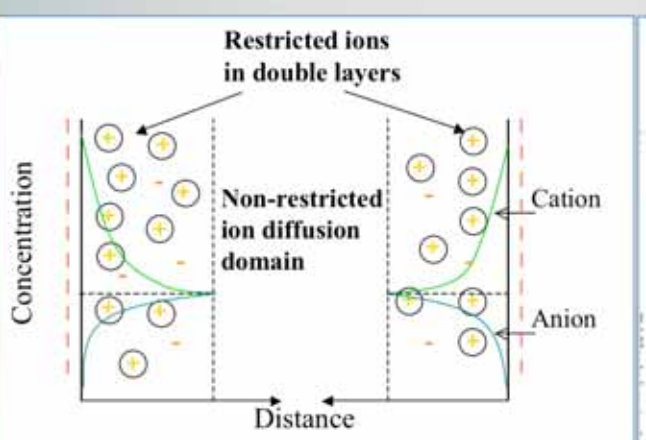
## Summary

- An NMR-PGSE technique indicated that a dual region diffusion model was required to simulate ion diffusion in the intragrain fractures of granitic lithic fragments in Hanford sediment.
- A new coupled model of electrostatics and nonequilibrium thermodynamics (EDNT) was developed to incorporate the effects of microscopic properties of complex ionic charge coupling, mineral surface charges, ion exchanges, and electrostatic double layers on ion diffusion.
- The rate of microbial reduction of intragrain U(VI) was determined by the sequential coupling of U(VI) dissolution, diffusion, and microbial activity. For MR-1, our results showed that U(VI) dissolution and diffusion out of intragrain regions were required before U(VI) could be microbially reduced.
- Microbial reduction of intragrain U(VI) in the contaminated Hanford sediment was complicated by the dissolution of calcite that released Ca to complex uranyl carbonates, which in turn slowed bioreduction rate. Not all intragrain U(VI) was bioavailable due to mass transfer limitation in poorly connected intragrain pores.

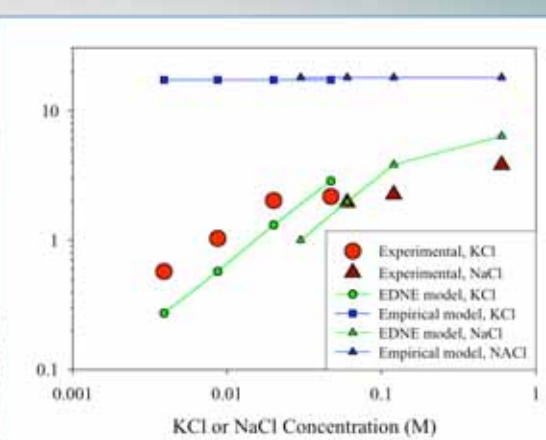
## Models of Ion Diffusion Coefficients

A coupled model of electrostatics and nonequilibrium thermodynamics (EDNT) was developed to calculate ion diffusivity in porous media. The model extends a commonly applied empirical expression:  $D_a = \tau D_w$  ( $D_a$  is the apparent diffusivity,  $D_w$  is the diffusivity in water, and  $\tau$  is the tortuosity), by including surface charges and electrostatic double layers. The new model indicated that apparent ion diffusivity in porous media is a complex function of surface charges, electrostatic double layer properties, electrolyte composition, chemical osmotic properties, hydraulic conductivity, and intrinsic tortuosity.

### Schematic diagram of ion diffusion regions



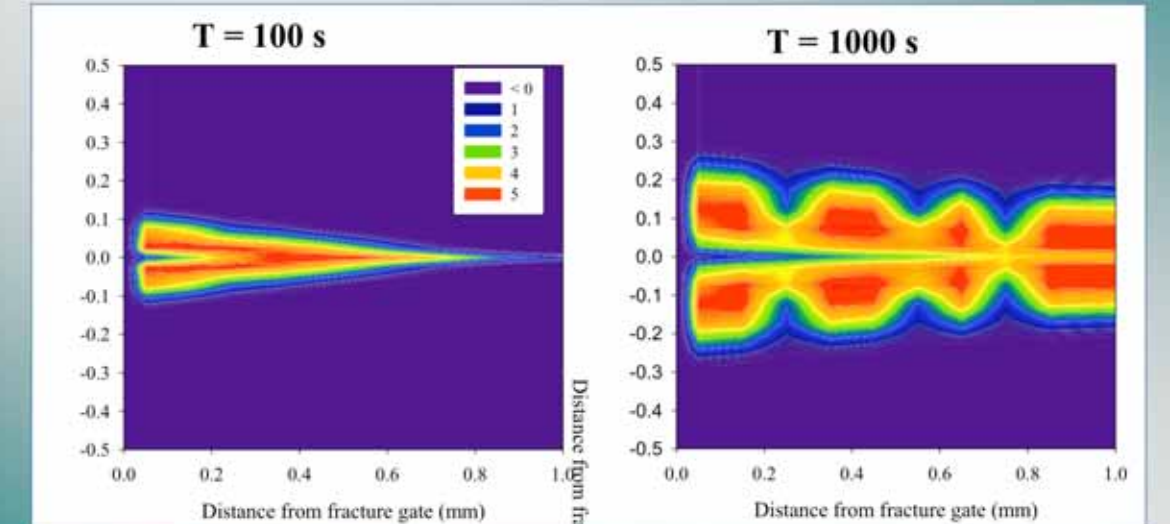
### Apparent KCl and NaCl diffusivity in clay materials



- Distribution of ions adjacent to a negatively charged surface;
- Electrostatic double layers restrict ion diffusion by decreasing ion diffusion rates and the free ion diffusion domain.
- Apparent KCl and NaCl diffusivities in clay materials decreased with decreasing ionic strength;
- Our model (EDNT) was able to calculate the effects of ionic strength, while the empirical diffusion model cannot.

## U(VI) Reactive Diffusion

A microscopic dual domain reactive diffusion model was developed to describe diffusive mass transfer phenomena.

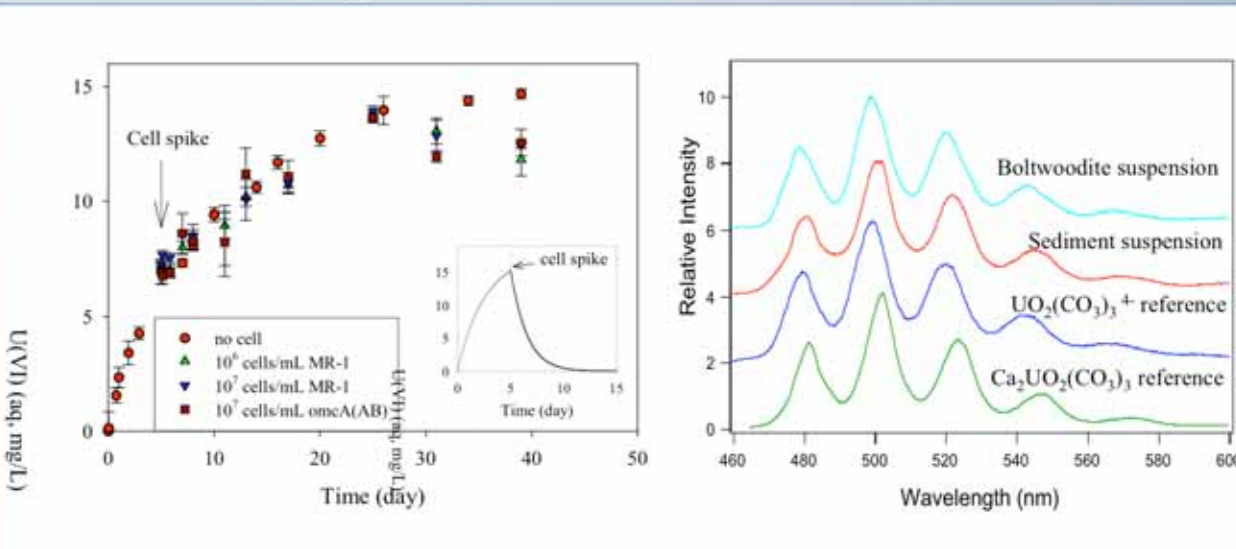


Simulated distributions of Na-boltwoodite saturation index (log IAP/K<sub>sp</sub>) in a single microfracture represented by a fracture and matrix system during U(VI) reactive diffusion from pore water.

## Microbial Reduction of U(VI) in Hanford Sediment

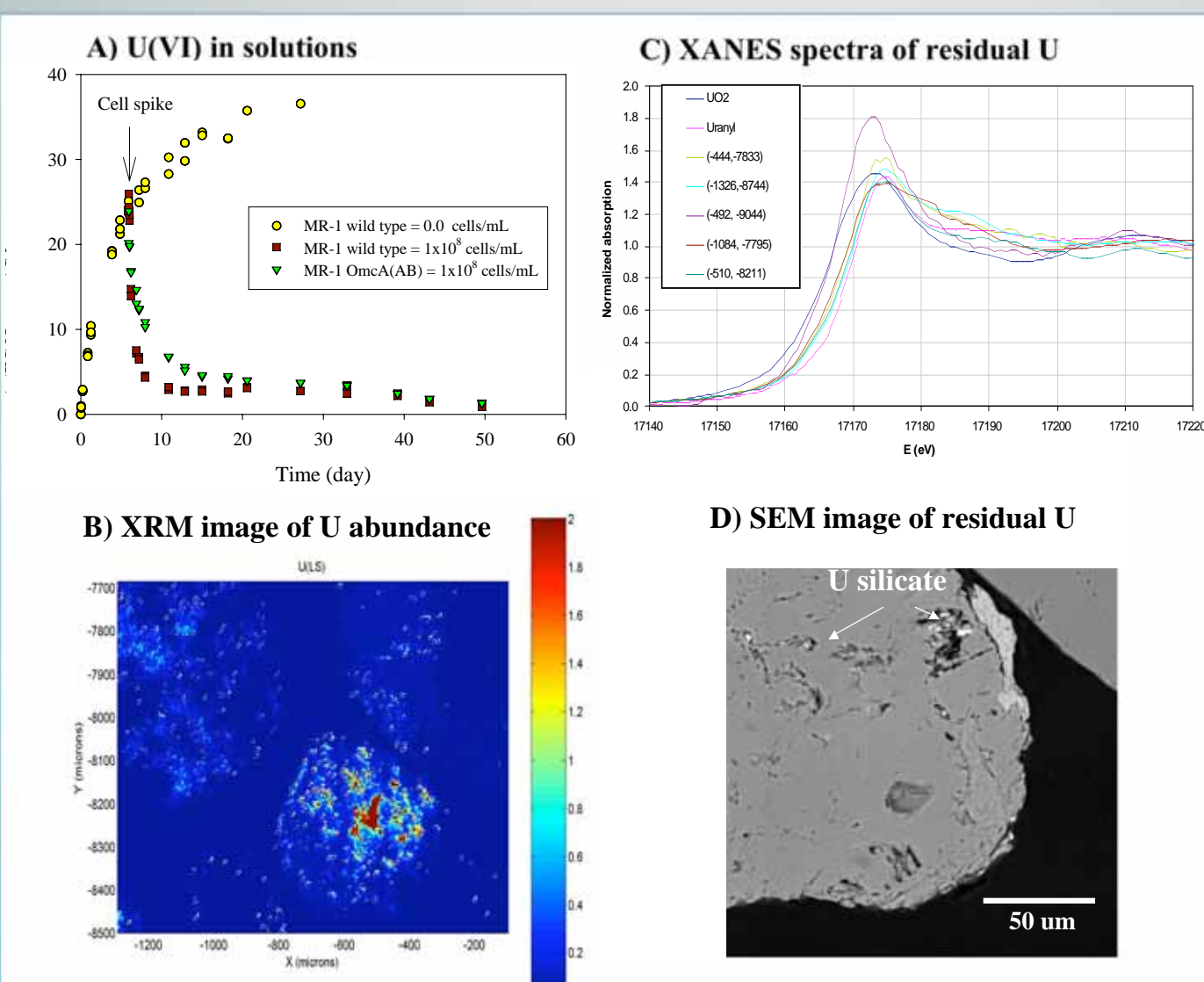
The accessibility of intragrain uranyl silicate precipitates in granitic lithic fragments was investigated to evaluate the applicability of the coupled model to contaminated sediment with more complex mineralogy.

### Effect of U(VI) aqueous speciation on microbial reduction rates



The bioreduction rates of U(VI) dissolved from contaminated sediment (left) were much slower than predicted from the dissolution rate of synthetic Na-boltwoodite (left inset). Although electrolyte without Ca<sup>2+</sup> was used in both experiments, LIFS analysis (right) showed that Ca<sub>2</sub>UO<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub> dominated aqueous U(VI) speciation in the sediment suspensions while UO<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub><sup>4-</sup> reference in the Na-boltwoodite suspension. These and other results indicated that Ca in the sediment suspensions was from calcite dissolution and Ca complexation with uranyl carbonate slowed the bioreduction rate.

### Bioavailability of intragrain U(VI)



- A: The coupling of dissolution, diffusion, and microbial reduction determined the aqueous U(VI) concentration in the sediment-MR-1 suspensions. A MR-1 deletion mutant devoid in outer membrane cytochrome OmcA, had a similar reduction rate to MR-1.
- B: The abundance of residual U within the sediment grains increased toward the grain center after microbial reduction for 60 days.
- C: XANES analysis indicated a mixed valence distribution of U(VI) and U(IV) inside the particle.
- D: SEM image showed the residual uranium in the pores that are enclosed or poorly connected to extragrain regions. Some U existed within the structure of zircon that was enclosed within the granitic lithic fragments.